Treatment of haemophilia and its complications

CHAPTER 6

Summary and prospectives

Replacement treatment is necessary for hemophiliacs throughout their life-spans and the complications associated with the use of the concentrates have an impressive impact on the clinical status of hemophiliacs. At this time, about 30% of Italian patients with severe hemophilia are HIV-infected (Ghirardini A. *et al.* National Haemophilia Registry, Italian Group for Congenital Coagulopathies: 1996 Report) and the prevalence is higher if only the adult population is considered. Almost all adult hemophiliacs are chronically infected with HCV. These serious blood-borne infections have not affected hemophilic children who have received safer replacement therapy. However, the occurrence of inhibitors is still the major complication for these children.

The work presented in this thesis was based on the experience at our Center with the clinical management of hemophilia and its heterogeneous complications and on research into this complex, interdisciplinary disease.

Chronic Hepatitis C

Currently, in many Hemophilia Centers the adult cohort mainly consists of patients with chronic hepatitis C but not infected with HIV, at high risk of liver-related morbidity and mortality. Antiviral treatment of HCV infection might be of clinical importance in this population. Our experience with interferon alpha therapy in hemophiliacs with chronic hepatitis C (Chapter 2) gave disappointingly low rates (13%) of sustained virologic responses, in spite of the prolongation of interferon treatment (3 MU three times a week) for up to 12 months. In agreement with the findings in other studies (Telfer *et al.* Thromb Haemost 1995; 74: 1259), we have identified epidemiologic and virologic factors in hemophiliacs that are associated with poor responsiveness, these being long duration of HCV infection, HCV genotype 1 and high viremia levels. These results have prompted a search for other therapeutic agents, such as ribavirine and amantadine. The results of a recent randomized trial in nonhemophilic patients (Reichard O. *et al.* Lancet 1998; 351: 83) have indicated that combination treatment with interferon and oral ribavirin may offer a better prospect of sustained virological response than interferon alone. We have employed the combined treatment with interferon/ribavirin for hemophiliacs previously unresponsive to interferon monotherapy and the encouraging results of this study, too preliminary to be included in this thesis, provide the basis for using this combined therapy for hemophiliacs not previously exposed to interferon.

The identification of noninvasive markers predicting the severity of the disease among hemophiliacs at risk for end-stage liver disease, indicating those most likely to benefit from treatment, is still a research priority. Given the natural history of chronic hepatitis, an increasing number of hemophiliacs will become candidates for liver transplantation. In these patients transplantation will allow correction of the underlying coagulation

defect, because both factor VIII and IX are synthesized by hepatocytes. The association of HCV infection with a number of extrahepatic manifestations has recently changed the prospective of the natural history of the disease. In this framework, the detection of cryoglobulinemia in about one third of our HCV-infected hemophiliacs (Chapter 2) raises concerns about the clinical significance of this complication. Interestingly, none of the patients had clinical signs of systemic vasculitis. Our data demonstrate that the risk of developing cryoglobulins increases with the duration of HCV infection, but the relevance of this HCV-related disorder still has to be established. A large prospective surveillance study is underway to address this issue, particularly with respect to the possible, long-term evolution towards non-Hodgkin lymphoma, currently being investigated for its association with HCV infection and cryoglobulinemia.

HIV infection

Prolonged survival of HIV-infected hemophiliacs has been observed in several series (Phillips AN. *et al.* Jama 1992; 268: 2662). In our cohort (Chapter 3), 10 years after seroconversion the cumulative survival was 76% and AIDS-free survival was 71%. Interestingly, HIV infection was more prevalent and associated with shorter survival in patients with hemophilia B, according to the national data provided by the Registry (Schinaia N. *et al.* Thromb Haemost 1994; 72: 33). Several factors, that influence positively survival and AIDS-free survival in hemophiliacs, have been recognised such as young age and high CD4 cell counts at seroconversion. Currently, the introduction of highly active anti-retroviral therapy (HAART) has further changed the figures for AIDS progression and also for survival. Nevertheless, limited group of patients have never required antiviral treatment to maintain normal CD4 lymphocyte counts. Patients with non-progressive HIV infection offer a unique model to study HIV pathogenesis. Long-term non-progressors (LTNPs) may be a heterogeneous group of patients. A strong immune response against HIV proteins by cytotoxic T lymphocytes and anti-HIV neutralizing antibodies, as well as low viral replication, have been reported in LTNPs from various risk categories by several authors (Cao Y. *et al.* N Eng J Med 1995; 332:201, Pantaleo G. *et al.* N Eng J Med 1995; 332: 209). At present, the nature of non-progressive HIV infection still remains elusive. Our study of a homogeneous group of hemophilic LTNPs (Chapter 3) showed that low HIV loads and low replication in peripheral blood (measured as proviral DNA in PBMCs and HIV-RNA in plasma and PBMCs) are the strongest correlates of non-progression. However, no absolute distinction can be made between LTNPs and progressors based exclusively on virologic parameters, supporting the view that there is a continuum between these two otherwise strikingly diverse outcomes of HIV infection. The recent discoveries concerning the suppressive effect of chemokines, produced by immune cells, on HIV replication

(Cocchi F. *et al.* Science 1995; 270: 1811) and the identification of chemokine receptors as the second receptor for HIV (Feng Y. *et al.* Science 1996; 272: 872) suggest there is a protective role of chemokines or their receptors during HIV infection. The hypothesis that protection might be related to the inability of HIV to infect target cells in the absence of the receptor is supported by the results showing homozygous mutations of the chemokine receptor in HIV-exposed but not infected individuals (Samson M. *et al.* Nature 1996; 382: 722). These findings have led us to study (Chapter 3) the 14 hemophiliacs of our cohort who have escaped HIV infection despite having been given a median of 166,000 U of contaminated concentrate in the period between 1980 and 1985. None of them showed homozygous mutations in the chemokine structural gene. Nevertheless, our data showing a higher production of chemokines in HIV-exposed but not infected hemophiliacs than in unexposed controls, whether hemophiliacs or not, provides evidence on their role as initial effector components of the cellular immune response. The protective direct effect of chemokines has been demonstrated by in vitro experiments showing that supernatant from PBMCs obtained from the exposed but not infected hemophiliacs inhibited HIV infection and the inhibitory effect was abolished by anti-chemokine antibodies. These findings support ongoing research into agents mimicking chemokines and/or blocking cell chemokine receptors. Demonstration of natural resistance to HIV infection encourages study of preventive vaccines able to block viral entry and subsequent spread to cells, providing a specific cytotoxic response.

Other pathogenic agents

Even though the new generation of hemophiliacs does not have to deal with these serious viral complications, the issue of surveillance is still alive, since absolute viral safety is still not achieved. Plasma-derived products are widely used and the awareness that they still transmit B19 parvovirus (Chapter 4) raises concern about other unknown agents with similar resistance to robust viral inactivation procedures. Recombinant coagulation factors are safer with respect to transmission of conventional viral infections. The next generation of recombinant products will not be processed or formulated with human or animal proteins, thereby minimizing the risk of transmission of agents of heterologous origin and of such non-viral pathogens as prions. Even though recombinant technology has offered the promise of unlimited supply, at the moment, worldwide demand for recombinant products for hemophilia treatment far exceeds supply.

Inhibitor development

Recombinant products are currently used in developed countries for replacement for hemophilic children, who are under strict surveillance for inhibitor risk. The development of inhibitors changes the therapeutic prospective for these patients, who require

immune tolerance treatment to eradicate antibodies and bypassing products to treat acute bleeding. Our work on the assessment of safety and efficacy of therapeutic options for high titer inhibitor patients is reported in Chapter 5. Early home treatment with recombinant FVIIa was evaluated in patients of our Center on a self-treatment regimen with fixed doses and with the patients instructed on how to assess treatment efficacy and to detect side effects. Self-administration of recombinant FVIIa at home proved to be of excellent feasibility and safety and resulted in a high success rate (79%) with a small number of doses (median 2), demonstrating the existence of a correlation between early treatment with recombinant FVIIa and effective outcome. Discordant data have been reported about the frequency and severity of side effects after the use of porcine factor VIII (Brettler DB. *et al.* Arch Intern Med 1989; 149: 1381, Hay CRM. *et al.* Blood 1990; 76: 882, Gringeri A. *et al.* Thromb Haemost 1991; 65: 245), making the safety of this treatment questionable. Different dose regimes and treatment modalities were employed in hospitalized patients and in the home setting, making comparison of data unreliable. An international survey (Chapter 5) was used to address this issue through retrospective data collection using a standardized questionnaire. The incidence of transfusion reactions was quantified after the use of porcine factor VIII as home infusions (0.001%), infusions in multiply treated in-patients (0.64%) and unselected in-patients infusions (2.3%). The low rate of side effects observed in patients treated at home was attributable to the low doses used by this group. The common post-infusion fall in platelet counts was usually transient and was judged to be clinically insignificant. Introduction of a viral inactivation step in the manufacturing process for porcine factor VIII is foreseen in the near future to further improve the safety profile of this preparation.

The observation of development of inhibitor in two adult brothers with mild hemophilia A led us to report (Chapter 5) this rare clinical condition, characterized by an unusual behaviour in response to replacement treatment, with anamnestic inhibitor rise after exogenous factor VIII administration and satisfactory response to desmopressin, suggesting a low immunogenicity of endogenous factor VIII. DNA analysis identified a missense mutation in the factor VIII gene. Subsequent data collection from US and European Centers (Chapter 5) has detected 26 patients with mild/moderate hemophilia A and inhibitors, confirming the association of this particular phenotype with relatively few missense mutations in the A2 and C2 domains.

The general characterization of anti-factor VIII inhibitors by their specific binding to epitopes mainly located in the A2 and C2 domains (Scandella D. *et al.* Pro Natl Acad Sci USA 1988; 85: 6152) has led to an experimental approach based upon the use of the recombinant hybrid human/porcine factor VIII molecule. This contains porcine substitutions at human epitope sites, avoiding at the same time the exposure of porcine

neoepitopes (Lollar P. Thromb Haemost 1999; 82: 505). Furthermore, epitope mapping is the basis for the development of recombinant, human factor VIII molecules with reduced immunogenicity.

The ultimate prospective in the cure of hemophilia is gene therapy. Hemophilia A and B are ideal diseases to be cured by gene therapy for several reasons, including the following: they are single-gene disorders, they require intravenous replacement treatment at frequent intervals, tight regulation of expression is probably unnecessary and tissue-specific expression is probably unimportant.
Considerable interest and enthusiasm have been generated in this research field in recent years, but formidable challeges remain. A variety of approaches have been developed, including viral and nonviral gene delivery systems and different target cells for gene expression. Adequate, sustained levels of factor VIII or IX have not been fully realized. In preclinical studies using hemophilic murine and canine models, immunogenicity resulting from the vectors and/or the new gene product has been cause for concern. Furthermore, there is at least the theoretical concern that certain viral vectors could potentially enter the germ line when administered intravenously. Recently, gene therapy trials have been started in humans with hemophilia aiming at a complete safety evaluation with careful, long-term follow-up. In addition, the issue of development of inhibitor in relation to gene therapy has to be addressed: will chronic exposure to antigen increase the risk of inhibitor or, alternatively, will it have a role to play in the treatment of patients with inhibitor?